Cat. # 7246

For Research Use

TaKaRa

Human Mitochondrial DNA (mtDNA) Monitoring Primer Set

Product Manual

v201409Da

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I. Description

Human Mitochondrial DNA (mtDNA) Monitoring Primer Set is designed to quantify the relative number of copies of human mitochondrial DNA (mtDNA) using nuclear DNA (nDNA) content as a standard by real-time PCR. This set contains primer pairs for the amplification of four regions: two primer pairs for the detection of mtDNA and two primer pairs for the detection of nDNA. Each primer is specifically designed and will not amplify pseudogenes. As the primers are human-specific and will not amplify DNA from mice, human mtDNA content can be monitored in iPS cells or other cells cultured with feeder cells of mouse origin.

Using the four primer pairs in this set for real-time PCR, relative quantification of mtDNA is determined from the difference in Ct values for mtDNA and nDNA. This product provides a simple method for monitoring changes in the mtDNA copy number as mtDNA has been reported to increase during differentiation of pluripotent cells, such as iPS cells, into hepatic cells.

The primer pairs in this set are compatible with Terra™ qPCR Direct SYBR® Premix (Cat. #638323/638319/638318) and SYBR *Premix Ex Taq*™ II (Tli RNaseH Plus) (Cat. #RR820A/B).

II. Components (50 Reactions)

1.	ND1 Primer Mix (10 μM each)＊1	50 μl
2.	SLCO2B1 Primer Mix (10 μM each)＊2	50 μl
3.	ND5 Primer Mix (10 μM each)＊1	50 μl
4.	SERPINA1 Primer Mix (10 μM each)＊2	50 μl

＊1: Primer for mitochondrial DNA (mtDNA)
＊2: Primer for nuclear DNA (nDNA)

III. Materials Required but not Provided

(Reagents)
- NucleoSpin Tissue (Cat. #740952.10/.50/.250)
- Terra qPCR Direct SYBR Premix (Cat. #638323/638319/638318) or SYBR *Premix Ex Taq*™ II (Tli RNaseH Plus) (Cat. #RR820A/B)

(Equipment)
- Micropipettes
- Micropipette tips (with hydrophobic filters)
- 0.2-ml 8-strip tube, individual Flat Caps (Cat. #NJ600)＊2

(Instruments)
- Real-time PCR amplification device＊1
  Thermal Cycler Dice™ Real Time System *II* (Cat. #TP900/TP960)＊2
  Thermal Cycler Dice Real Time System *Lite* (Cat. #TP700/TP760)＊2
  Thermal Cycler Dice Real Time System Single (Cat. #TP850/TP870)＊2
- Spectrophotometer

＊1: When the Applied Biosystems StepOnePlus Real-Time PCR System (Life Technologies) is used, it may be necessary to set the threshold manually to obtain a flat baseline.

＊2: Not available in all geographic locations. Check for ability in your region.

IV. Storage -20℃

※ Use within 1 year.
※ When using repeatedly within a short period of time (～1 month), store at 4℃. However, be careful not to introduce contamination, as the product does not contain any preservatives.

V. Protocol

Note:

To ensure proper use of reagents and equipment other than this product, refer to the user manual for each product.

Precautions:

Because mitochondrial DNA (mtDNA) is present in higher copy number than nuclear DNA (nDNA) and is smaller in size, there is a high possibility of mtDNA contamination in samples. It is important to protect samples from sources of human DNA contamination.

Use preventative measures, such as cleaning the working area and equipment with DNA-OFF™ (Cat. #9036) or another solution for the prevention of DNA contamination. Also pay careful attention during PCR setup; change gloves regularly and wipe pipettes with a cleaner such as ethanol or DNA-OFF. Additionally, using an ultraviolet sterilization cabinet for PCR setup is an effective means of preventing contamination from equipment, etc.

Workflow

Extract genomic DNA (including mtDNA) from target cells
Prepare highly purified DNA using NucleoSpin Tissue, etc.
↓
Prepare master mix for real-time PCR and dispense into PCR reaction tubes
↓
Add Primer Mixes
↓
Perform real-time PCR
Real-time PCR analysis of two mtDNA genes and two nDNA genes
↓
Analyze data
Relative quantification of the copy number of mtDNA using nDNA as the standard

For analysis, the file mtDNA_Copy_Number_Calculation.xls can be used to calculate mtDNA copy number using Microsoft Office Excel. The file is available for download from the product page on our website.

PCR Reaction

Four reactions are performed for each DNA sample (one reaction each of the four primer pairs).
Refer to the reaction composition given below to prepare a master mix for the number of tubes needed. The master mix should contain the genomic DNA template, but should not contain the Primer Mix. Dispense 24 μl of the master mix into each tube and then add 1 μl of Primer Mix. Preparation of the reaction mixture using this procedure minimizes the amount of variation due to pipetting errors in the amount of template dispensed and provide more reliable results.

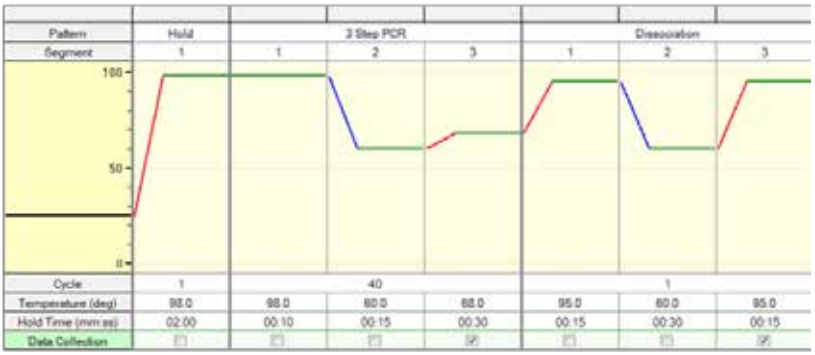
【When using the Thermal Cycler Dice Real Time System *II*】
※Operate according to the instrument user manual.

1. Prepare the reaction mixture below on ice.
   <Per Reaction>

	Volume	Final Concentration
Terra qPCR Direct SYBR Premix (2X) or SYBR *Premix Ex Taq* II (Tli RNaseH Plus) (2X)	12.5 μl	1X
Each Primer Mix (10 μM each)	1 μl	0.4 μM each
Genomic DNA sample (5 ng/μl)*	2 μl	
dH_2O (Sterile Distilled Water)	9.5 μl	
Total	25 μl	

*: Use 1 - 100 ng of genomic DNA per reaction.
Use highly purified DNA prepared using a purification kit such as NucleoSpin Tissue.
After purification, measure the OD_{260}/OD_{280} ratio of the genomic DNA to confirm purity.

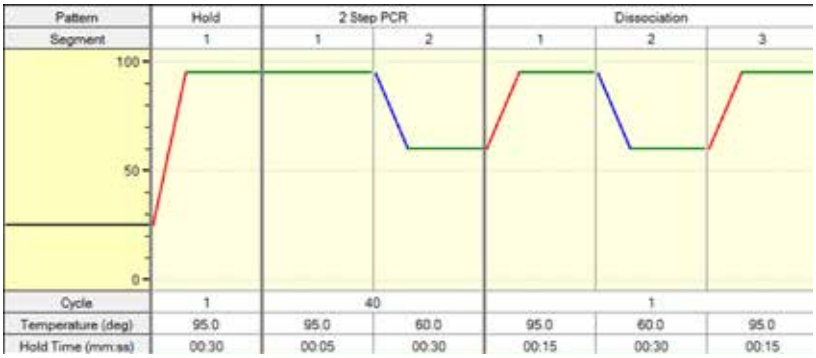
2. Start the reaction using the conditions indicated below.
   <When using the Terra qPCR Direct SYBR Premix>



Hold (Initial denaturation)
Cycle : 1
98℃ 2 minutes
3 Step PCR
Cycle : 40
98℃ 10 seconds
60℃ 15 seconds
68℃ 30 seconds
Dissociation

※ Precautions for Use
Terra qPCR Direct SYBR Premix includes an antibody for hot start PCR. Initial denaturation at 98℃ for 2 minutes must be performed to denature the antibody.

<When using the SYBR *Premix Ex Taq* II (Tli RNaseH Plus)>



Hold (Initial denaturation)
Cycle : 1
95℃ 30 seconds
2 Step PCR
Cycle : 40
95℃ 5 seconds
60℃ 30 seconds
Dissociation

3. The reaction is complete.
Perform data analysis and determine the mtDNA copy number by relative quantification.
Calculate the copy number of mtDNA from the Ct values obtained for each of the four target genes.
Follow the procedures below. The copy number of mtDNA is calculated as the average of the values calculated based on the combinations of the primers as indicated below.

Note: The Microsoft Office Excel file mtDNA_Copy_Number_Calculation.xls＊1 can be used for the calculation of the mtDNA copy number by inputting the Ct values for each primer.
This file can be used to analyze up to 5 experimental replicates and will calculate the average mtDNA copy number.

Calculation:

a. Determine the difference in the Ct values for the ND1/SLCO2B1 pair. (ΔCt1 = Ct for SLCO2B1 - Ct for ND1)
b. Determine the difference in the Ct values for the ND5/SERPINA1 pair in the same manner. (ΔCt2 = Ct for SERPINA1- Ct for ND5)
c. Find $2^{\Delta Ct}$ for the values for ΔCt1 and ΔCt2.
d. Use the average of the 2 values found in step c. as mtDNA copy number.

Example of the Calculation of Copy Number

Primer	Ct Value	ΔCt	$2^{\Delta Ct}$	Mean Copy Number of mtDNA
ND1	15.75	ΔCt1 = 8.78	440	574
SLCO2B1	24.53			
ND5	15.04	ΔCt2 = 9.47	709	
SERPINA1	24.51			

＊1 : Download from the product page on our website.

VI. Experimental Example

Relative quantification of the copy number of mtDNA in two types of human iPS cells and liver tissue was performed using genomic DNA templates (including mtDNA).

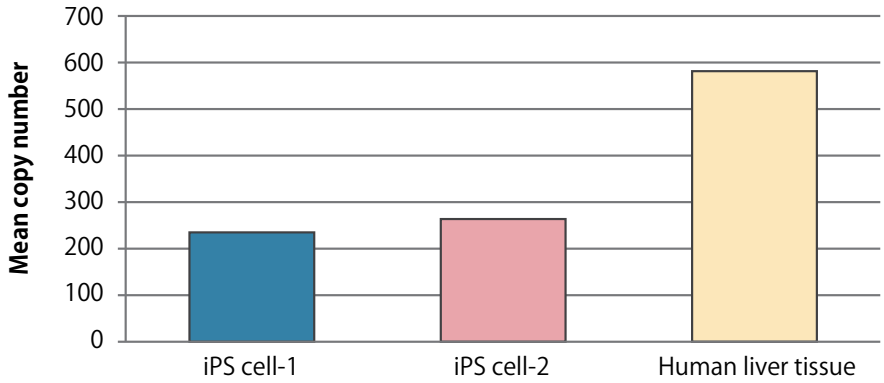
(Method)

Detection of mtDNA was carried out according to the protocol. Refer to "V. Protocol" for the reaction conditions and the method for calculating the copy number of mtDNA.

(Results)

The copy number of mtDNA has been reported to increase during differentiation of pluripotent cells, such as iPS cells, into hepatic cells. This experiment demonstrated that the mtDNA copy number in liver tissue, which is mostly comprised of hepatic cells, is greater than that of pluripotent cells.

Sample	Primer	Ct Value	ΔCt	$2^{\Delta Ct}$	Mean copy number
iPS cell-1	ND1	17.19	7.17	144	231
	SLCO2B1	24.36			
	ND5	16.17	8.31	317	
	SERPINA1	24.48			
iPS cell-2	ND1	17.08	7.37	165	260
	SLCO2B1	24.45			
	ND5	16.19	8.47	355	
	SERPINA1	24.66			
Human liver tissue	ND1	15.75	8.78	440	574
	SLCO2B1	24.53			
	ND5	15.04	9.47	709	
	SERPINA1	24.51			



Comparison of the mtDNA Copy Number

VII. Troubleshooting

If no amplification is observed during real-time RT-PCR

- The quantity of mtDNA may be unusually small due to the tissue, strain, and culture conditions of the cells. Optimize the quantity of template DNA used within the range of 1 - 100 ng as necessary.
- The use of genomic DNA (including mtDNA) of extremely low purity is not recommended. Use DNA that has been isolated using a purification kit such as NucleoSpin Tissue.
- Perform DNA extraction using the same method for all samples. Preparing using a different method may cause great differences in purity and affect the results.
- Prepare the real-time PCR reaction solution on ice and perform PCR as soon as possible. When on leaving the assembled reaction on ice, shield from light until the start of the reaction.

VIII. Reference

Yue, Yu, *et al*. (2012) Hepatocyte-like cells differentiated from human induced pluripotent stem cells: Relevance to cellular therapies. *Stem Cell Research* **9**:196-207.

IX. Related Products

NucleoSpin Tissue (Cat. #740952.10/.50/.250)
NucleoSpin Tissue XS (Cat. #740901.10/.50/.250)
Terra™ qPCR Direct SYBR® Premix (Cat. #638323/638319/638318)
SYBR® *Premix Ex Taq*™ II (Tli RNaseH Plus) (Cat. #RR820A/B)
Real Time PCR Equipment
Thermal Cycler Dice™ Real Time System *II* (Cat. #TP900/TP960)*
Thermal Cycler Dice™ Real Time System Single (Cat. #TP850/TP870)*
Thermal Cycler Dice™ Real Time System *Lite* (Cat. #TP700/TP760)*
0.2 ml 8-strip tube, individual Flat Caps (Cat. #NJ600)*
96 well Hi-Plate for Real Time (Cat. #NJ400)*
Sealing Film for Real Time (Cat. #NJ500)*
DNA-OFF™ (Solution for elimination of DNA contamination) (Cat. #9036)

*: Not available in all geographic locations. Check for availability in your region.

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